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DEGRADATION OF PESTICIDES BY ALGAE

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DEGRADATION OF PESTICIDES BY ALGAE

by

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ABSTRACT

In this investigation interactions of 12 pesticides with 37 strains of fresh water algae were studied in an effort to determine something of the variability in responses of fresh water algae to the variety of pesticides in use or projected to be used in the future.

Three interactions were investigated. One was the toxicity of the pesticides to these algae. Another was the sorption of several of the pesticides by some of the species of algae. The third was the possibility that some of the pesticides can be degraded by action of algae.

In general it was found that sensitivity of algae to pesticides varied greatly with the strains tested.

Sorption of methoxychlor appeared to be mainly physical, since much of the methoxychlor sorbed was exchangeable. The butoxyethyl ester of 2,4-D (2,4-DBE) was not sorbed to a significant extent by two green algae tested, and sorption of carbaryl was very slow.

Malathion can be degraded by algae in the presence of light. One breakdown product, malathion monoacid (beta form), appeared as the malathion was being degraded, and later disappeared. Investigations of the fate of 2,4-DBE and methoxychlor in algal cultures suggest that these pesticides may also be degraded by algal activity.

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ACKNOWLEDGMENTS

Appreciation is expressed to the companies donating the pesticides used in these studies and to J. F. Thompson, National Environmental Research Center, Research Triangle Park, North Carolina, for analytical standards. Special thanks are due M. H. Woolford and R. C. Blinn, American Cynamid Company, for standards of malathion products.

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Work carried out by graduate students Gary L. Butler and Sam W. Moss is included in this report.

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SECTION I

INTRODUCTION

Most prior studies of the interactions of pesticides and fresh water algae have dealt with a specific pesticide or a few, and have involved one or only a few species or strains of algae. Generally the algae employed have been common laboratory strains rather than organisms recently isolated from streams, lakes or ponds. The freshwater algae, as a group, are very heterogeneous, physiologically as well as morphologically. Because of this, predictions of pesticide interaction with algae in streams based upon such laboratory studies may be subject to significant error.

In addition, most of the studies carried out so far have been concerned only with the toxicity of pesticidesto algal species or strain. Only a few exceptions have considered other aspects of pesticide-alga interaction.

The present study is an attempt to broaden the base upon which predictions can be made about the interactions of fresh water algae and pesticides in nature. It also considers, in addition to sensitivity of algae to pesticides, sorption of pesticides and algal activities leading to pesticide breakdown. It involved 36 strains of algae isolated from the Warrior River near Tuscaloosa, Alabama and one additional laboratory strain, Anacystis nidulans.

The pesticides investigated included: aldrin, atrazine, captan, 2,4-DBE (the butoxy ethyl ester formulation), diazinon, dieldrin, endrin, heptachlor, malathion, methoxychlor and toxaphene.

SECTION II

CONCLUSIONS AND RECOMMENDATIONS

While the toxicity of the different pesticides used in this study varies significantly according to the pesticide involved, each pesticide inhibited growth in one or several of the Warrior River isolates at concentrations of a few parts per million or less. The sensitivity to a particular pesticide varied significantly with different isolates. For this reason, it is concluded that estimates of environmental damage to the flora of streams and other bodies of fresh water are prone to error if they are based only upon studies of the effects of pesticides on a few laboratory strains of algae. It is recommended that such estimates be based in the future upon studies that utilize algae representative of those native to a particular aquatic environment.

Of the pesticides tested in this study atrazine was by far the most toxic to the Warrior River algae. Some of these organisms, which are much more sensitive to atrazine than has been reported previously, can be inhibited by atrazine levels as low as 10^{-3} mg/l. Thus it is recommended that more extensive studies of atrazine in the aquatic environment be carried out.

Sorption of methoxychlor, 2,4-DBE, and carbaryl varied significantly with different strains and species of fresh water algae. The ^{14}C-methoxychlor rapidly taken up by these organisms was also rapidly exchanged with unlabeled methoxychlor. Thus, it is concluded that much of the uptake was physical adsorption to cell surfaces. Some strains of fresh water algae lack binding sites for 2,4-DBE and consequently do not take it up in detectable quantities. A long lag in the uptake of ^{14}C-carbaryl by some fresh water algae suggests that these organisms only take up breakdown products of this pesticide and not the parent compound. These limited sorption studies lead to the conclusion that generalizations about uptake of pesticides by algae may lead to errors in estimations of environmental effects of such uptake.

A biological breakdown of malathion mediated by fresh water algae can occur in the presence of light. It also appears that some fresh water algae can degrade methoxychlor and 2,4-DBE even though the latter may not be absorbed. It is recommended that additional studies of these phenomena be conducted in order to obtain better estimates of the fates of these pesticides in the aquatic environment.

SECTION III

MATERIALS AND METHODS

PESTICIDES AND PESTICIDE DERIVATIVES

The unlabeled pesticides used were: 1) aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanonaphthalene)(analytical standard, 99.5%) supplied by Shell Chemical Company; 2) atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine)(99.0%) supplied by Aldrich Chemical Co., Inc.; 3) captan (N-(trichloromethyl thio)-4-cyclohexene-1,2-dicarboximide)(MP 173-174°C) supplied by Matheson, Coleman and Bell; 4) carbaryl (1-naphthyl N-methylcarbamate) (99.7%) supplied by Union Carbide Corporation; 5) 2,4-DBE (2,4-dichlorophenoxyacetic acid, butoxy ethyl ester)(98.2%) supplied by Amchem Products, Inc.; 6) dieldrin (1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo, exo-5,8 dimethanonaphthalene) (analytical standard, 99.5%) supplied by Shell Chemical Company; 7) diazinon (0,0-diethyl-0-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate)(99.9%) supplied by CIBA-GEIGY Corporation; 8) endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo, endo-5,8 dimethanonaphthalene)(analytical standard, 99.5%) supplied by Shell Chemical Company; 9) heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene)(purity 99.4% by labile chlorine) supplied by Velsicol Chemical Corporation; 10) malathion (0,0-dimethyl S-(1,2-dicarbethoxy ethyl phosphorodithioate)(purity 99.3%) supplied by American Cyanimid Company; 11) methoxychlor (2,2-bis (p-methoxyphenyl)-1,1,1-trichloroethane)(88.0% p-methoxyphenyl isomer, 12.0% other isomers) supplied by E. I. DuPont; 12) toxaphene (chlorinated camphene compounds of uncertain identity, combined chlorine 67-69%) supplied by Hercules, Inc.

The labeled pesticides used included ^{14}C-carbaryl (ring-labeled), American Radiochemical Corporation; methoxychlor-(ring-UL-^{14}C), Mallinckrodt; and 2,4-dichlorophenoxyacetic acid-1-^{14}C-(butoxy ethyl ester formulation), New England Nuclear.

Analytical standards for gas chromatography were provided by J. F. Thompson, Chief, Quality Assurance Section, Chemistry Branch, EPA, Pesticides and Toxic Substances Effects Laboratory, National Environmental Research Center, Research Triangle Park, N. C., 27711.

The following derivatives of malathion were provided by M. H. Woolford, Jr. and R. C. Blinn, American Cyanimid: malaoxon, malathion dicarboxylic acid, malathion monocarboxylic acid (beta-form), potassium

dimethyldithiophosphate, potassium dimethylthiophosphate and potassium-O-desmethyl malathion.

ALGAL ISOLATION AND MAINTENANCE OF STOCK CULTURES

The algae utilized in this study (except for one culture strain) were isolated from water samples collected from the Black Warrior River at Bennett's Marina boat landing just above the Hugh Thomas Bridge at Tuscaloosa, Alabama. Water samples were collected in sterile 1 liter bottles and returned to the laboratory where the algae were isolated. Some of the samples were used for isolations on the day of collection and some samples were enriched by mixing with equal volumes of double strength medium. Three media were used for enrichment: 1) Bristol's Inorganic Mineral Solution (Deason and Bold, 1960), 2) Diatom Medium FW-1 (Lewin, 1966), and 3) Blue-green Algal Medium BG-11 (Stanier *et al.*, 1971). The enriched samples were incubated 1-2 weeks to allow the algal populations to increase in number. Following the incubation period the algae were isolated.

Isolations were made from the original samples and enriched cultures by plating and spraying (Pringsheim 1946; Wiedeman *et al.*, 1964). Axenic cultures were obtained by successive plating and spraying of the unialgal cultures. All cultures were examined periodically for contamination by inoculating from stock cultures into microbiological media (nutrient broth, nutrient broth + 1.0% w/v glucose) and by direct microscopic observations.

The isolated algae were maintained on slants of FW-1 medium (Lewin, 1966) in 18 x 150 mm test tubes capped with plastic caps. FW-1 was also used as the culture medium following the initial isolations. All stock cultures were maintained in a walk-in growth chamber on a 16-8 hr light-dark cycle at 20 ± 1°C. Illumination of 3500 lux was provided by Westinghouse cool-white fluorescent tubes.

Since the isolates did not include a blue-green alga several large-scale experiments utilized a laboratory strain of *Anacystis nidulans*, strain B 625 from the Indiana University Culture Collection of Algae (Starr, 1964), which was maintained in stock as a liquid culture in the BG-11 medium of Stanier *et al.* (1971). These stocks were maintained with the isolate stocks and under the same environmental conditions.

Media used for maintaining stock cultures, and all other media used, were sterilized by autoclaving for 15 min. at 121°C and 105.5 g/cm^2.

ALGAL TOLERANCE OF PESTICIDES

All pesticide tolerance (algal growth in the presence of pesticide) experiments were conducted using 18 x 150 mm test tubes capped with plastic caps. The pesticides tested were aldrin, atrazine, captan, carbaryl, 2,4-DBE, dieldrin, diazinon, endrin, heptachlor, malathion, methoxychlor and toxaphene.

A quantity of each pesticide was weighed out and dissolved either in pesticide grade acetone or hexane (Burdick & Jackson, Inc.). Varying amounts of the pesticide/solvent mixtures were added to liter quantities of sterilized FW-1 medium to give the desired concentration. Since preliminary tests showed that the concentrated pesticides and solvents did not contain bacterial contaminants, the pesticide/solvent mixtures were not sterilized.

Following the addition of the pesticide to the medium, it was stirred overnight to provide sufficient time for the pesticide to dissolve and the solvent to evaporate. The concentration of the solvent used was 0.5% or less, a concentration which preliminary experiments showed did not affect algal growth. Using aseptic technique, 10 ml of pesticide-containing medium was pipetted into sterile test tubes.

The algae to be used for inocula were transferred from 4-week-old agar slants to 50 ml FW-1 medium in 125-ml Erlenmeyer flasks and the flasks were then placed on a shaker in the growth chamber. Liquid cultures 4-6 days old were used for inocula and all such cultures were in the logarithmic phase of growth. Each test tube was inoculated with a standardized inoculum and then placed in the growth chamber. Cultures were neither aerated nor shaken. At the end of a 2-week growth period the cultures were removed, agitated on a Vortex-Genie mixer, and growth was measured turbidimetrically using a Klett-Summerson colorimeter equipped with a red filter.

PESTICIDE LOSS IN SMALL ALGAL CULTURES

The cultures for the pesticide loss studies were prepared using the procedures described for the pesticide tolerance experiments. The initial concentration of pesticide in the cultures was 1 mg/l for atrazine, diazinon and malathion, and 0.01 mg/l for methoxychlor and 2,4-DBE.

At the end of the 2-week growth period the cultures were extracted along with control tubes containing pesticide and medium but no algae and the extracts were analyzed for loss of pesticide.

The pesticide in each 10-ml culture was extracted using a 80:20 v/v mixture of pesticide grade benzene and hexane (Burdick & Jackson, Inc.). Five milliliters of the solvent mixture was added to each test tube and then agitated thoroughly on a Vortex-Genie mixer. The layers were allowed to separate, and with a disposable Pasteur pipette, the solvent phase was removed. Each culture was extracted twice and the two solvent portions combined. Sufficient anhydrous sodium sulphate was added to take up any water present, and the solvent was transferred to a graduated centrifuge tube.

The extracts were reduced to a working volume (1-10 ml) using a gentle stream of air and then analyzed for pesticide content using a Tracor MT-220 Gas-Liquid Chromatograph equipped with a Nickel-63 electron capture detector.

The column used was a 6 mm OD x 4 mm ID x 1.83 m glass column containing Chrom W HP 80/100 mesh support coated with 3% OV-1 (Varian Aerograph).

Analysis conditions for atrazine, diazinon, methoxychlor and 2,4-DBE were:

Carrier gas flow (N_2)	120 ml/min
Detector temperature	250°C
Inlet temperature	225°C
Column temperature	
Atrazine, diazinon	145°C
Methoxychlor	180°C
2,4-DBE	165°C

Analysis conditions for malathion were:

Carrier gas flow (N_2)	120 ml/min
Detector temperature	240°C
Inlet temperature	195°C
Column temperature	170°C

Calculations to quantitate concentration in a sample involved triangulation of the peaks printed out for the pesticides atrazine, diazinon, methoxychlor and 2,4-DBE. For malathion an Autolab model 6300 Digital Integrator was used.

LARGE SCALE CULTURE CONDITIONS

In order to provide relatively large quantities of algal cells, four 10-liter continuous cultures of several organisms were maintained axenically in culture apparatus that has been described (O'Kelley, 1966). Illumination for these cultures was provided by Westinghouse

cool-white fluorescent tubes at a culture surface intensity of 8.7 x 10^4 ergs/cm^2-sec as determined with a YSI-Kettering radiometer. Cultures were stirred continuously with magnetic stirring bars, and 1% CO_2 in air was bubbled through the cultures, except for Anacystis nidulans cultures, which were aerated only with air. Cultures were continuously maintained in a logarithmic stage of growth by periodically draining the culture and adding fresh, sterile medium.

The culture medium for green algal isolates was Bristol's Inorganic Mineral Solution (Deason and Bold, 1960); for isolates of the diatom Nitzschia,it was medium ASP-2, modified (Mann and Meyers, 1968); and for Anacystis nidulans,it was medium BG-11 (Stanier et al., 1971).

PESTICIDE UPTAKE AND EXCHANGE BY ALGAE

The pesticides used in these studies included both ^{14}C-labeled and unlabeled forms of methoxychlor, carbaryl and 2,4-DBE described previously. The initial concentration of pesticide in these experiments was 0.01 mg/l methoxychlor, or 1 mg/l 2,4-DBE, or 1 mg/l carbaryl. Organisms studied include Chlorella sp. (isolate #1), Nitzschia sp. (isolate #35), Monoraphidium sp. (isolate #11) and Anacystis nidulans.

In order to study uptake of one of these pesticides by algal cells, 350 ml of medium containing the proper concentration of labeled pesticide was prepared. In the case of methoxychlor this was stirred for 24 hours to insure dissolution. A 350 ml aliquot of liquid algal culture was then removed from a 10 liter continuous culture, and a turbidimetric reading was made using a Klett-Summerson colorimeter equipped with a red filter. Except in the case of Nitzschia cultures, the cells from the 350 ml were then collected by centrifugation at 9750 x g in a Sorvall RC2-B refrigerated centrifuge, resuspended in 2.0 ml of medium and added to the 350 ml of stirring medium containing labeled pesticide. Cells from the diatom cultures were collected on 5.0 μ pore size Millipore filters (Millipore Corporation), and then scraped off the filters. Diatom cells were then resuspended in 2.0 ml of growth medium and added to the 350 ml of stirring medium containing labeled pesticide. Medium and cells were stirred, aerated and illuminated under the same conditions as for the 10 liter continuous cultures. Three 10 ml samples were then taken from the stirring culture at prescribed times periods following the addition of cells. Samples were collected on glass fiber filters (Reeve Angel 934AH, Arthur Thomas Company) to collect the cells (for Nitzschia and green algae) or ultra fine glass fiber filters (Reeve Angel 984H, Arthur Thomas Company) for Anacystis. Filters with cells were dried at room temperature for eight hours and cut up into small pieces in polyethylene scintillation vials (Primavials, Nuclear Associates) containing 10.0 ml of a scintillation cocktail. The cocktail was prepared by mixing 500 ml of Triton X-100

(New England Nuclear), 917 ml of toluene (Eastman Chemicals) and 83 ml of Liquifluor (New England Nuclear). A standard quantity (0.4 g) of Cabosil thixotropic gel (Beckman Instruments) was added to each vial to suspend pieces of filters, and samples were counted for 10 minutes each in a Packard Tri Carb Liquid Scintillation Counter.

Radioactivity counting standards were prepared as follows: Cells from the 10 liter continuous culture at the same turbidity as experimental cultures were collected on a glass fiber filter. The filter was dried and cut up into a scintillation vial containing 10 ml of scintillation cocktail, 0.4 grams of Cabosil and a standard quantity of labeled pesticide.

By passing 10 ml of medium containing labeled pesticide but no algae through glass fiber filters, the radioactivity held by the filters was determined. This value was subtracted from counting rates obtained from each cell sample in order to obtain counts associated only with the cells. By comparing the counts of each sample with counts obtained with the standard, the total number of nanograms of pesticide associated with the cells was determined. Finally, utilizing a cell turbidity reading (indicating cell density) all pesticide uptake was expressed as nanograms of pesticide per gram (wet weight) of algal cells.

In order to determine whether or not the labeled pesticide taken up by the cells could be exchanged with unlabeled pesticide at various time intervals after adding cells to the medium containing labeled pesticide, some of these cells were re-collected and re-suspended in 10 volumes of medium containing the same concentration of unlabeled pesticide. Samples were then taken at prescribed time intervals from the suspension of labeled cells in medium containing unlabeled pesticide and the amount of labeled pesticide still associated with the cells was determined as before. This was expressed as nanograms per gram (wet weight) of algal cells. The amount of labeled pesticide that exchange with unlabeled pesticide was then calculated, and the quantity of initial radioactivity in the cells that was lost by exchange was determined.

MALATHION DEGRADATION IN LARGE SCALE CULTURES

The organisms used in this study were Chlorella sp. (isolate #1) Nitzschia sp. (isolate #35) and Anacystis nidulans.

These studies involved 4-liter cultures prepared as were the 10-liter cultures previously described in this report. Cultures were utilized for an experiment when the cell density for Chlorella and Anacystis reached 4 g/l and for Nitzschia reached 0.9 g/l. At this

point malathion, dissolved in acetone, was added axenically to give an initial malathion concentration in the culture of 1 mg/l. Culture conditions remained the same as before the malathion addition except that aeration for all organisms was by air, rather than by 1% CO_2 in air. A control was set up that was treated similarly, but included medium and malathion and no algal cells. After addition of the malathion, samples were taken immediately (time zero), after 1 hr, 4 hr, 8 hr, 12 hr, and then at 12 hr intervals until 144 hr. Ten-ml samples were taken in triplicate and extracted as described earlier for quantitative determination of malathion by gas chromatography. Fifty-ml samples were extracted by the following procedure: The 50 ml sample of algal culture containing pesticide was placed in a 250 ml separatory funnel and extracted twice with 100 ml chloroform (technical grade, Fisher). Following the extractions sufficient anhydrous sodium sulfate was added to remove water. After separating and washing the sodium sulfate with an additional 10 ml of chloroform the chloroform extract was evaporated to 1 ml using vacuum or a slow stream of air. The 1 ml samples were then used to spot thin-layer chromatographic plates.

The extracts condensed to 1 ml were then co-chromatographed on thin layer plates with the following standards: malathion, malaoxon, malathion dicarboxylic acid, malathion monocarboxylic acid (beta-form), potassium dimethyl dithiophosphate, potassium dimethylthiophosphate and potassium-O-desmethyl malathion. Silica gel 60 (E. Merck) plates were equilibrated in a glass chamber and developed using hexane:acetic acid:ether (75:15:10) as the solvent system (Kadoum, 1970). Following development the plates were air-dried and then sprayed with 2,6-dibromo-N-chloro-p-quinoneimine (Menn. *et al.*, 1957). Following the spraying, the plates were heated 10 min at 110°C and examined.

In several experiments using the Chlorella strain, at the time malathion was added, the culture was placed in darkness where it was kept until all samples were taken. In one experiment when the Chlorella cell density reached 4 g/l the medium was separated from the cells by continuous flow centrifugation and the experiment was conducted under illumination but using only the supernatant, without algal cells.

SECTION IV

RESULTS

ISOLATION OF ALGAE USED IN THE STUDY

A number of algae were isolated from the Warrior River water samples and 36 isolates were obtained in unialgal axenic culture (Table 1). Subsequent study of the isolates revealed that some were replicates of the same morphological and physiological strain. These replications are indicated in Table 2, which also shows that the 36 isolates represent 21 different strains of algae.

TOLERANCE TO PESTICIDES BY ALGAL ISOLATES

Interpretation of Tabulated Results

Tables 3 and 4 show the response of the Warrior River algae (not specifically identified) to varying levels of pesticides in the culture medium. The vertical columns of each table indicate algal growth as a range of the percent of growth of the same organism with no pesticide present; the horizontal rows in each table indicate pesticide concentration. The figures in the body of each table show the number of isolates that grew to a certain percentage range, relative to the growth of the same isolate without any pesticide. For example, in Table 3, in 0.01 mg/l carbaryl, five isolates were stimulated to grow to a level 111-150% of the control growth; 12 isolates grew approximately as well in this concentration of pesticide as they did in control tubes (91-110%), and the growth of 19 isolates was inhibited (51-90% of controls) in medium containing 0.01 mg/l carbaryl.

Aldrin

Aldrin effects were measured on the 21 strains of Warrior River algae (Table 4). A majority of the strains were inhibited by concentrations of 0.01 and 0.1 mg/l. Seven of the strains were inhibited more than 50% by a concentration of 1.0 mg/l; in contrast to this, two strains appeared to be slightly stimulated by this concentration of aldrin.

Table 1. LIST OF ALGAE ISOLATED FROM THE WARRIOR RIVER

1. Chlorella sp.	19. Carteria sp.
2. Chlorella sp.	20. Chlorella sp.
3. Golinkiniopsis sp.	21. Scenedesmus sp.
4. Chlorella sp.	22. Chlorella sp.
5. Chlorella sp.	23. Koliella sp.
6. Chlorella sp.	24. Chlorella sp.
7. Chlorella sp.	25. Chlorella sp.
8. Chlorella sp.	26. Chlorella sp.
9. Chlorella sp.	27. Chlorella sp.
10. Chlorella sp.	28. Scenedesmus sp.
11. Monoraphidium sp.	29. Scenedesmus sp.
12. Actinastrum sp.	30. Scenedesmus sp.
13. Chlorella sp.	31. Nitzschia sp.
14. Koliella sp.	32. Nitzschia sp.
15. Chlorella sp.	33. Nitzschia sp.
16. Chlorella sp.	34. Nitzschia sp.
17. Carteria sp.	35. Nitzschia sp.
18. Chlorella sp.	36. Nitzschia sp.

Table 2. DIFFERENT MORPHOLOGICAL AND/OR PHYSIOLOGICAL STRAINS REPRESENTED BY THE 36 ISOLATES FROM THE WARRIOR RIVER

Strain	Isolate Number
Chlorella sp.	1,5,8,27
Chlorella sp.	2,26
Golinkiniopsis sp.	3
Chlorella sp.	4
Chlorella sp.	6,24,25
Chlorella sp.	7
Chlorella sp.	9,10,13
Monoraphidium sp.	11
Actinastrum sp.	12
Koliella sp.	14,23
Chlorella sp.	15
Chlorella sp.	16
Carteria sp.	17,19
Chlorella sp.	18
Chlorella sp.	20
Scenedesmus sp.	21
Chlorella sp.	22
Scenedesmus sp.	28,29,30
Nitzschia sp.	31,35,36
Nitzschia sp.	32,34
Nitzschia sp.	33

Table 3. NUMBER OF DIFFERENT ISOLATES OF THE WARRIOR RIVER ALGAE, IN CULTURES CONTAINING PESTICIDE, THAT GREW TO A CERTAIN PERCENTAGE RANGE, AS COMPARED TO GROWTH IN CONTROL CULTURES (NO PESTICIDE)

Pesticide	Concentration mg/l	Growth Range, Per Cent of Controls				
		0-50	51-90	91-110	111-150	151-190
Atrazine	0.001	2	24	10	--	--
	0.01	--	31	5	--	--
	0.1	5	16	15	--	--
	1.0	32	--	4	--	--
	10.0	36	--	--	--	--
	25.0	36	--	--	--	--
Carbaryl	0.01	--	19	12	5	--
	0.1	--	14	18	4	--
	1.0	--	18	18	--	--
	10.0	4	10	12	10	--
	25.0	15	4	5	12	--
2,4-DBE	0.001	--	8	12	13	3
	0.01	--	5	15	13	3
	0.1	--	5	11	15	5
	1.0	--	5	13	14	4
	4.0	--	16	9	7	4
Diazinon	0.01	1	30	4	1	--
	0.1	--	9	7	12	8
	1.0	--	10	10	10	6
	10.0	7	22	6	--	1
	25.0	21	9	3	2	1
Methoxychlor	0.001	--	9	12	13	2
	0.01	--	13	11	8	4

Table 4. NUMBER OF DIFFERENT STRAINS OF THE WARRIOR RIVER ISOLATES, IN CULTURES CONTAINING PESTICIDE, THAT GREW TO A CERTAIN PERCENTAGE RANGE, AS COMPARED TO GROWTH IN CONTROL CULTURES (NO PESTICIDE).

Pesticide	Concentration mg/l	Growth Range, Per Cent of Controls				
		0-50	51-90	91-110	111-150	151-190
Aldrin	0.01	3	14	4	--	--
	0.1	2	12	5	2	--
	1.0	7	8	4	2	--
Captan	0.01	--	8	12	1	--
	0.1	--	6	10	5	--
	1.0	--	15	6	--	--
	5.0	3	8	8	2	--
	8.0	4	13	3	1	--
Dieldrin	0.01	3	13	3	2	--
	0.1	2	12	6	1	--
	1.0	7	9	4	1	--
Endrin[a]	0.01	--	8	11	1	--
	0.1	2	14	3	1	--
	1.0	4	14	1	1	--
Heptachlor[a]	0.01	--	14	5	1	--
	0.1	--	10	7	2	--
	1.0	2	11	4	1	1
Malathion	1.0	--	9	8	4	--
	5.0	--	7	9	4	1
	10.0	--	12	4	4	1
	25.0	12	6	2	1	--
	50.0	17	4	--	--	--
Toxaphene	0.001	--	12	6	3	--
	0.01	--	2	13	5	1
	0.1	1	7	8	5	--
	1.0	8	7	6	--	--

[a] One strain of Nitzschia, of the 21 strains, was not tested.

Atrazine

Of all pesticides studied, atrazine was by far the most inhibitory to the algal isolates (Table 3). Of the 36 isolates, 26 were measurably inhibited by 0.001 mg/l atrazine; two isolates at this concentration were inhibited more than 50%. Inhibition was slightly more severe at 0.01 mg/l and 0.1 mg/l. At 1 mg/l atrazine all 36 isolates were inhibited and 32 of these grew at a rate less than 50% of that of the controls. At 10 mg/l inhibition was even more severe, and at 25 mg/l all isolates grew at a rate less than 50% of that in the control tubes.

Captan

Captan inhibited several strains (of the 21 tested) slightly at a concentration of 0.01 mg/l (Table 4). Inhibition increased progressively as the captan concentration was increased, up to at least 8.0 mg/l. At a concentration of 5.0 and 8.0 mg/l approximately half of the strains were inhibited more than 50%, compared to controls with no captan.

Carbaryl

Overall, the growth of the 36 isolates was about as good in 0.01 and 0.1 mg/l carbaryl as was observed in the controls (Table 3). Some organisms appeared to be stimulated slightly while others were slightly inhibited. However, at 1 mg/l carbaryl no isolates were stimulated and 18 were inhibited. At 10 mg/l carbaryl 4 isolates were severely inhibited (less than 50% of control growth), but the growth of 10 isolates was stimulated by this pesticide. At 25 ppm 15 isolates were inhibited such that their growth was 50% (or less) of growth in control cultures; again the response of other isolates was quite different in that 12 isolates were measurably stimulated by this level of carbaryl.

2,4-DBE

At 0.001, 0.01, 0.1 and 1.0 mg/l 2,4-DBE approximately as many isolates were stimulated by the herbicide as were inhibited by it (Table 3). At 4 mg/l, the highest level tested, more isolates were inhibited (16 isolates) than stimulated (11 isolates). The isolates inhibited at this level of 2,4-DBE grew at a rate more than 50% of that of the controls, and the 4 isolates most stimulated had grown significantly more than did the controls (151-190% of growth in the controls).

Dieldrin

Inhibition of the algal strains by dieldrin was similar to that observed for aldrin (Table 4). Seven strains were inhibited more than 50% by 1 mg/l dieldrin. A few strains appeared to be slightly stimulated, rather than inhibited, by dieldrin at concentrations not higher than 1.0 mg/l.

Diazinon

Diazinon, at the lowest concentration tested, 0.01 mg/l, inhibited the growth of 31 of the isolates, but only one isolate was inhibited more than 50%; growth was stimulated for one isolate (Table 3). In contrast, at 0.1 and 1.0 mg/l, diazinon appeared to stimulate growth of approximately half of the isolates. At 10 mg/l only 1 isolate appeared to be stimulated, and 29 isolates were measurably inhibited. At 25 ppm growth of 30 of the 36 isolates was measurably inhibited, and growth of 21 of these isolates was less than 50% of that of the controls.

Endrin

Toxicity of endrin to the algal strains tested appeared to be somewhat lower than that of aldrin and dieldrin (Table 4). Eight strains showed some inhibition at an endrin level of 0.01 mg/l. However, only four strains were inhibited 50% or more by 1.0 mg/l endrin. One strain was stimulated by endrin at all three concentrations tested.

Heptachlor

Heptachlor showed limited toxicity to the algal strains isolated from the Warrior River (Table 4). Fourteen strains showed some inhibition, however, at 0.01 mg/l. While a concentration of 0.1 mg/l appeared to be no more inhibitory, 1.0 mg/l was more toxic to two strains. A few of the strains of algae were stimulated by these concentrations of heptachlor.

Malathion

Malathion was sparingly toxic to the algal strains tested at concentrations of 1.0 and 5.0 mg/l (Table 4). About half of the strains were inhibited slightly, nearly half were unaffected, and several were stimulated. A few more strains (12) were inhibited at 10.0 mg/l. At 25 and 50 mg/l malathion most or all of the algal strains were inhibited significantly.

Methoxychlor

Since methoxychlor is sparingly soluble in aqueous solutions, it was added to algal cultures only at levels of 0.001 and 0.01 mg/l (Table 3). At these levels, methoxychlor inhibited the growth of about one-third of the 36 isolates, about one-third had their growth unaffected by methoxychlor, and approximately one third of the isolates were stimulated to grow by methoxychlor.

Toxaphene

The pattern of response of twenty algal strains to toxaphene was as follows: at 0.001 mg/l 12 strains were inhibited, but growth was more than 50% of that of the controls (Table 4). At 0.01 mg/l toxaphene a number of the algal strains were stimulated. Slightly more inhibition was observed at 0.1 mg/l. At a concentration of 1.0 mg/l eight of the strains were inhibited more than 50%; one strain did not grow in this concentration of toxaphene.

PESTICIDE LOSS IN SMALL-SCALE ALGAL CULTURES

2,4-DBE

Loss of this formulation of 2,4-D in small scale algal cultures was high in all organisms tested (Table 5). Isolate #18, a Chlorella sp., retained the highest quantity of 2,4-DBE relative to the control tubes, 53%, while isolate #17, a Carteria sp., removed all but 13% of the 2,4-D relative to control tubes.

Methoxychlor

These culture experiments indicate that algae will remove methoxychlor from the environment (Table 5). The most active organism in this respect was a Nitzschia species (isolate #32), and of the green algal species tested, Chlorella (isolate #1) was most active. The values of methoxychlor in culture tubes, expressed as the percentage of methoxychlor in control tubes at the end of the two week growth period, ranged from 29 to 79.

Malathion

As in the case of methoxychlor and 2,4-D, malathion was lost from all of the small scale cultures more rapidly than from control tubes lacking algae (Table 5). The most active organism for removing meth-

Table 5. PESTICIDES RECOVERED FROM ALGAL CULTURES AFTER 2-WEEK GROWTH PERIOD, AS PERCENTAGE OF PESTICIDE IN CONTROLS

ISOLATE NUMBER	ATRAZINE	2,4-DBE	DIAZINON	METHOXYCHLOR	MALATHION
1.	91	33	96	34	47
2.		17	94	37	19
3.		35	82	63	58
4.	99	13	90	65	42
6.		24	62	42	42
7.	99	27	82	63	69
10.	98	41	80	65	43
11.	98	64	78	40	35
12.		20	78	40	44
15.	104	18	88	63	44
16.	104	24	89	76	36
17.		13	78	55	25
18.	104	53	94	67	63
20.	102	44	91	79	74
21.		25	83	37	35
22.	104	18	86	69	41
23.	98	51	87	61	75
28.	107	25	80	42	46
31.		17	77	57	38
32.		14	75	29	41
33.		22	85	44	77

oxychlor was a Chlorella sp. (isolate #2) and the least active was a Nitzschia sp. (isolate #34). The values for pesticide lost in cultures as a result of algal presence ranged in percentage from 23 to 81.

Diazinon

Loss of diazinon in 2-week small scale cultures (Table 5) was lower than was loss of 2,4-DBE, methoxychlor or malathion. Only isolate #6, a Chlorella sp., was effective; in cultures of this organism, pesticide loss was 61% of the pesticide in control tubes at the end of the 2-week period. Less than 25% was removed by the other 20 strains.

Atrazine

Atrazine loss (Table 5) did not occur significantly in small scale algal cultures containing this pesticide. This may have resulted because of the high toxicity of atrazine to these isolates (see Table 3); algal growth was meager in the presence of atrazine and, perhaps, for this reason the algae did not degrade the atrazine significantly.

SORPTION OF LABELED PESTICIDES

Methoxychlor

Results of the ^{14}C-methoxychlor sorption experiments are seen in Table 6. Four organisms were used in the study, two green algae (Chlorella sp., isolate #1; Monoraphidium sp., isolate #11), one diatom (Nitzschia sp., isolate #35), and one blue-green alga (Anacystis nidulans). For each organism, there was a rapid initial sorption of methoxychlor, indicative of physical adsorption. For Chlorella (isolate #1), this rapid sorption involved approximately 7% of the pesticide initially added to the medium; for Monoraphidium (another green alga), the rapid sorption involved about 13% of the pesticide in the medium; for Anacystis, it involved about 7% of the pesticide; for Nitzschia (isolate #35), it involved about 84% of the total pesticide available for sorption. In Monoraphidium and Chlorella there was a slower sorption, as represented by increased radioactivity associated with the cells up to 6 hr after beginning the experiment. For all of the organisms the label held by the algal cells diminished after 6 hr. The medium was counted to see if the label was being excreted back into it, but counts revealed that medium activity did not increase concomitant with loss of label within the cells. The loss may represent conversion of the ^{14}C in methoxychlor to $^{14}CO_2$.

Table 6. SORPTION OF ^{14}C-METHOXYCHLOR BY FRESH-WATER ALGAL SPECIES

Time of Sorption	[a]Amount of ^{14}C-Methoxychlor taken up by indicated organism, ng per g fresh weight			
	Chlorella (Isolate #1)	Monoraphidium (Isolate #11)	Nitzschia (Isolate #35)	Anacystis nidulans
1 min	214	799	5205	399
5 min	426	762	--	424
10 min	397	731	--	415
15 min	--	--	5642	--
20 min	378	764	--	390
30 min	--	--	5558	--
1 hr	367	836	5528	383
3 hr	278	--	5687	--
6 hr	259	1063	5704	428
12 hr	155	--	--	391
18 hr	--	--	5097	--
24 hr	22	645	--	316

[a] The initial concentration of methoxychlor was 0.01 mg/l. The values reported are the means from duplicate experiments involving cells from two different cultures of the same organism. The initial cell density for all organisms except Nitzschia was 4 g wet weight per liter of medium; for Nitzschia it was 0.9 g/l Volume of all cultures was 500 ml.

2,4-DBE

Results of the ^{14}C-2,4-DBE sorption experiments are shown in Table 7. Organisms utilized were Chlorella sp. (isolate #1), Nitzschia sp. (isolate #35) and Anacystis nidulans. Of the three organisms tested only the diatom, Nitzschia, took up appreciable quantities of this formulation of 2,4-D. The rapid nature of this sorption indicated that it was physical adsorption. The label was rapidly lost from the cells after its initial sorption. The other two organisms possess few or no binding sites for this form of 2,4-D; a very small, but detectable quantity was taken up by the Chlorella isolate and this was soon lost. There was no detectable sorption of 2,4-DBE by Anacystis nidulans.

Carbaryl

In contrast to ^{14}C-methoxychlor and ^{14}C-2,4-DBE, ^{14}C-carbaryl did not appear to be taken up rapidly by any of the three organisms studied (Chlorella sp., isolate #1; Nitzschia sp., isolate #35; Anacystis nidulans) (Table 8). Sorption of the label by Nitzschia was approximately linear for the first three hours, after which time the quantity of label held by the cells remained essentially unchanged for 24 hr. In contrast, for Chlorella, there was no detectable sorption of label for 3 hr and the maximum sorption rate was seen between 3 and 6 hr after the ^{14}C-carbaryl was introduced. With Anacystis there was no detectable sorption at 12 hr after exposure of the cells to ^{14}C-carbaryl, but the cells accumulated some of the label after 12 hr.

EXCHANGE OF LABELED PESTICIDES

Results of the methoxychlor exchange experiments are shown in Table 9. In Chlorella sp. (isolate #1) and in Anacystis nidulans after 5 minutes of methoxychlor sorption, about 90% will exchange with unlabeled methoxychlor, indicating that 90% is adsorbed physically to the cell surface; after 6 hr of methoxychlor sorption 90% can still be exchanged. This indicates that little methoxychlor has been taken up metabolically by the protoplasts of the cells. In the case of Monoraphidium (isolate #11) after 5 min. sorption, 80% will exchange with non-radioactive methoxychlor. However, after 6 hr sorption only 55% will exchange; this indicates that 45% has been taken up by the protoplasm or has been degraded. The uptake behavior of Nitzschia, isolate #35, is more difficult to interpret. Even after a short sorption period of 5 min. much of the methoxychlor is not exchangeable. Specifically, after 5 min. sorption, only 40% will exchange with non-labeled pesticide offered the cells. The value after 6 hr uptake decreases slightly, with only 35% exchanging.

Table 7. SORPTION OF ^{14}C-2,4-DBE BY FRESH-WATER ALGAL SPECIES

Time of Sorption	[a]Amount of ^{14}C-2,4-DBE taken up by indicated organism, µg per g fresh weight		
	Chlorella (Isolate #1)	Nitzschia (Isolate #35)	Anacystis nidulans
1 min	2.6	193.6	0
5 min	-	195.2	0
10 min	0.4	-	0
20 min	0	174.8	0
1 hr	0	127.9	0
3 hr	0	81.7	0
6 hr	0	18.0	0
12 hr	0	7.7	0
24 hr	0	4.8	0

[a] The initial concentration of 2,4-DBE was 1 mg/1. The values reported are the means from duplicate experiments involving cells from two different cultures of the same organism. The initial cell density of all organisms except Nitzschia was 4 g wet weight per liter of medium; for Nitzschia it was 0.9 g/1.

Table 8. SORPTION OF ^{14}C-CARBARYL BY FRESH-WATER ALGAL SPECIES

Time of Sorption	[a]Amount of ^{14}C-carbaryl taken up by indicated organism, µg per ml fresh weight		
	Chlorella (Isolate #1)	Nitzschia (Isolate #35)	Anacystis nidulans
1 min	0	-	0
5 min	-	0.4	-
10 min	0	1.2	0
20 min	0	2.1	0
1 hr	0	3.4	0
3 hr	0.3	7.8	0
6 hr	0.3	8.2	-
12 hr	6.4	7.3	-
24 hr	2.4	6.8	14.9

[a] The initial concentration of carbaryl was 1 mg/l. The values reported are the means from duplicate experiments involving cells from two different cultures of the same organism. The initial cell density for all organisms except Nitzschia was 4 g wet weight/of medium; for Nitzchia it was 0.9 g/l.

Table 9. EXCHANGE OF ^{14}C-METHOXYCHLOR FROM ALGAL CELLS TO MEDIUM CONTAINING UNLABELED METHOXYCHLOR

Isolate #	Organism	Uptake Time	Exchange Time	Per Cent Exchanged	Per Cent Not-exchanged
1	Chlorella sp. (Isolate #1)	5 min	5 min	75	25
			1 hr	89	11
			6 hr	88	12
		6 hr	5 min	72	28
			1 hr	93	7
			6 hr	92	8
	Anacystis nidulans	5 min	5 min	87	13
			1 hr	93	7
			6 hr	95	5
		6 hr	5 min	87	13
			1 hr	88	12
			6 hr	92	8
11	Monoraphidium sp. (Isolate #11)	5 min	5 min	83	17
			1 hr	88	12
			6 hr	88	12
		6 hr	5 min	43	57
			1 hr	54	46
			6 hr	58	42
35	Nitzschia sp. (Isolate #35)	5 min	5 min	33	67
			1 hr	42	58
			6 hr	40	60
		6 hr	5 min	25	75
			1 hr	35	65
			6 hr	34	66

The sorption of 2,4-DBE was negligible for all organisms tested except for Nitzschia, isolate #35. Thus the only exchange experiments using 2,4-DBE involved this isolate. Table 10 shows that the labeled pesticide taken up by this organism in 5 min was rapidly exchanged with unlabeled pesticide.

The peculiar pattern of ^{14}C-carbaryl sorption by the three algal strains tested, namely the long delay in uptake of the ^{14}C label, was such that exchange experiments did not appear to be appropriate for any of the algal strains being studied.

MALATHION DEGRADATION

Large scale algal cultures were utilized to provide a large enough sample of algal cells to permit a sequential study in the laboratory of malathion breakdown caused by the action of algal cells. Three organisms, two of them indicated to be active in breaking down malathion in test tube cultures, were selected; they were Chlorella sp. (isolate #1), Nitzschia sp. (isolate #35) and Anacystis nidulans.

Two of these organisms, under illumination, were very active in breaking down malathion (see Table 11); one was the Chlorella isolate and the other was Anacystis nidulans; malathion breakdown was very rapid compared to the no-cell controls. In contrast, the Nitzschia isolate showed lower activity. Each experiment had a no-cell control consisting of medium with equivalent initial malathion concentration and subjected to similar physical conditions (temperature, light, aeration) to the extent possible. Malathion breakdown without algae varied somewhat in different experiments, but in all of these controls the malathion half-life was calculate to be 110 hr or longer.

Since the Chlorella isolate appeared the most active in breaking down malathion, its activity was studied in more detail. In one experiment both the Chlorella culture and its no-alga control were placed in darkness, rather than in light. While there was measurable breakdown of malathion by Chlorella in the dark, as compared to malathion breakdown in the dark control, the dark rate was very much lower than the malathion breakdown rate in illuminated Chlorella cultures (see Table 12). In another experiment, a Chlorella culture was grown to the cell density desired and the medium was separated from the cells by refrigerated continuous flow centrifugation. Then malathion was added to illuminated supernatant, and its fate was followed. Malathion breakdown was negligible in the illuminated supernatant for 24 hr. At 36 hr the experiment was terminated since the culture was becoming green from the renewed production of Chlorella cells from a few that had not been removed by centrifugation.

Table 10. EXCHANGE OF ^{14}C-LABELED-2,4-DBE USING NITZSCHIA SP. (ISOLATE #35)

Uptake Time	Exchange Time	% Exchanged	% Not exchanged
5 min	5 min	16	84
	1 hr	4	96
	6 hr	5	95
6 hr	5 min	[a]50	[a]50
	1 hr	[a]50	[a]50
	6 hr	[a]50	[a]50

[a] The quantity of label present on cells after 6 hr exposure to labeled pesticide was only 2 per cent of that present after 5 min exposure; 50% values represent only 1% of that initially taken up by the Nitzschia cells.

Table 11. [a]MALATHION CONCENTRATION IN LARGE-SCALE CULTURES OF FRESH-WATER [b]ALGAE. VALUES ARE PER CENT OF INITIAL MALATHION CONCENTRATION

Time, Hours	Chlorella (Isolate #1)	Nitzschia (Isolate #35)	Anacystis nidulans	Control (no algae)
0	100.0	100.0	100.0	100
1	97.6	95.1	98.0	--
2	98.9	92.9	97.3	97.3
4	83.9	90.9	94.6	--
8	29.0	72.9	87.2	88.2
12	12.2	63.0	60.0	--
24	0	59.8	32.7	85.2
36	--	59.8	14.5	--
48	0	56.5	0	84.3
60	--	54.9	--	--
72	0	50.1	0	82.0
84	--	44.3	--	--
96	0	41.8	0	77.2
108	--	40.1	--	--
120	0	34.7	0	75.5

[a] Initial malathion concentration was 1 mg/l.

[b] Initial algal cell concentration was 4 g/l medium for Chlorella and Anacystis; for Nitzschia it was 0.9 g/l.

Table 12. COMPARISON OF [a]MALATHION CONCENTRATION IN LARGE-SCALE CULTURES CONTAINING ILLUMINATED CHLORELLA (ISOLATE #1) CELLS WITH CONCENTRATION IN DARKENED CHLORELLA CULTURE, AND IN "AGED" MEDIUM CENTRIFUGED TO REMOVE CELLS AND ILLUMINATED

Time, Hours	Chlorella (Illuminated)	Chlorella (Dark)	Chlorella Medium (Illuminated)
0	100.0	100.0	100.0
1	97.6	--	--
2	98.9	96.7	85.0
4	83.9	--	--
8	29.0	74.1	71.1
12	12.2	--	75.5
24	0	65.8	70.0
36	--	--	[b]21.1
48	0	65.8	--
60	--	61.2	--
72	0	60.3	-
84	--	57.1	--
96	0	51.1	-
108	--	46.5	--
120	0	46.0	-
132	--	43.3	--
144	0	38.2	--

[a] Initial malathion concentration 1 mg/l.

[b] Culture had become green from the production of new Chlorella cells from the small number remaining after centrifugation.

Plots of the logarithm of malathion concentration in these cultures against time indicated that after a brief lag period (2 to 4 hr) malathion disappearance approximated that expected from a first order reaction. Therefore the process was treated as a pseudo-first-order reaction in these cultures, and in controls, in order to obtain approximate malathion degradation rates and malathion half-lives in the cultures. Duplicate rate constants and half-lives obtained for Chlorella and for Anacystis, as well as for degradation of malathion in the controls, are shown in Table 13.

Samples from the Chlorella culture represented in Table 11 were extracted, and the extracts were placed on thin-layer chromatographic plates along with standards of malathion degradation products. Malathion monocarboxylic acid was found first in the 8-hr sample, reached a maximum concentration in the 12-hr sample and was not found in the 24-hr samples or later. Other degradation products were not identified.

Table 13. MALATHION DEGRADATION RATE CONSTANTS AND PESTICIDE HALF-LIVES FOR ILLUMINATED CULTURES (CALCULATED AS A PSEUDO-FIRST-ORDER PROCESS)

Organism	Replicate	k	$T_{1/2}$, hr
[a]*Chlorella* (Isolate #1)	1	0.454	1.54
	2	0.241	2.89
[a]*Anacystis nidulans*	1	0.044	15.9
	2	0.064	10.8
Control (no algae)	1	0.006	116.4
	2	0.004	240.2

[a] Cell concentration 4 g wet weight/l of culture medium.

SECTION V

DISCUSSION

ALDRIN AND DIELDRIN

Dieldrin is toxic to the alga Agmenellum quadruplicatum at concentrations of 0.475 and 0.95 mg/l while aldrin is not; these concentrations of dieldrin are non-toxic to this species and also to Anacystis nidulans (Batterton et al., 1971). Poorman (1973) reported that there was stimulation, rather than inhibition of growth of Euglena gracilis by 10, 50 and 100 mg/l of aldrin. Growth of the diatom Navicula seminulum was reduced 10% by 1.8 mg/l dieldrin and was stopped by 32 mg/l (Cairns, 1968). Stadnyk et al. (1971) found a 10% decrease in the cell number of Scenedesmus quadricaudata at 0.1 and 1 mg/l dieldrin after 10 days of exposure. In contrast to the tolerance of some of these organisms to aldrin and dieldrin, growth of a number of the Warrior River isolates was inhibited by these insecticides at 0.01, 0.1 and 1.0 mg/l. In-so-far as accumulation is concerned, Wheeler (1970) found that Chlorella pyrenoidosa accumulated dieldrin, and Rice and Sikka (1973) reported that marine diatoms could remove dieldrin from culture media.

ATRAZINE

The effect of atrazine on several laboratory strains of algae has been studied previously. Ashton et al. (1966) reduced the growth of Chlorella vulgaris 85% by treating with 70 mg/l for 72 hours. Atkins and Chan (1967) also reported inhibition to a Chlorella sp., but found that the inhibition was overcome by the organism and growth equalled that of the controls after 12 days. Zweig et al. (1963) reported a 50% inhibition in O_2 evolution by Chlorella pyrenoidosa using 6.2 mg/l atrazine. Wells and Chappel (1965) found that a concentration of 0.1 mg/l atrazine was only slightly inhibitory to a thermophilic strain, Chlorella pyrenoidosa 7-11-05. Both Wells and Chappell (1965) and Kratky and Warren (1971) found that concentrations of 1 mg/l and higher were severely inhibitory. Arvik et al. (1971) found that 0.73 mg/l atrazine reduced growth of an unidentified soil alga by about 50%. Gramlich and Frans (1964) observed a 70% reduction of growth of Chlorella pyrenoidosa by 1.25×10^{-8} M atrazine. Less than 2.5 mg/l reduced growth of a Chlorella-like alga by 95% (Kruglov, 1970). Loeppky and Tweedy (1969) found that the effect of atrazine varied depending upon the alga involved, some species being inhibited completely by 0.5 mg/l. Walsh (1972) obtained similar results with four unicellular marine algae. Our studies with the Warrior River isolates demonstrate that some aquatic algae are much more sensitive to atrazine than has been

reported previously and that organisms can be inhibited by atrazine levels as low as 10^{-3} mg/l.

The Warrior River algae were severely inhibited by atrazine at 1 mg/l; there was no atrazine loss in cultures that could be attributed to action by these algal species.

CAPTAN

Soeder et al. (1973) found that the amount of growth inhibition caused by captan was dependent upon the algal strain being studied; while 37 strains of Chlorella were strongly inhibited by 2.5 to 5 mg/l captan, three strains of Chlorella and two strains of Scenedesmus were tolerant of 50 mg/l. Moore (1967) found that the blue-green alga Nostoc muscorum was not inhibited by 1 mg/l. Similarly, the Warrior River isolates were not inhibited by 1 mg/l captan. However, at 5 and 8 mg/l captan growth was inhibited in about one-quarter of these isolates.

CARBARYL

Prior studies of interactions of carbaryl and algae have been concerned mainly with toxicity of carbaryl. Carbaryl at 0.1 and 1.0 mg/l stimulates growth of some algae and inhibits others (Ukeles, 1962, Stadnyk et al. 1971). Higher concentrations (100 mg/l) kill some organisms (Ukeles, 1962) while others are reported to be inhibited only slightly (Butler, 1963; Christie, 1969). Carbaryl inhibited growth of the Warrior River isolates at concentrations of 0.01, 0.1 and 1.0 mg/l. As the concentration was increased to 10 and 25 mg/l, growth of several isolates was stimulated. Because the non-biological breakdown of carbaryl is rapid, this stimulation may have been from a breakdown product or products. Carbaryl appears to be taken up readily by the Nitzschia sp. (isolate #35); however, neither the Chlorella sp. (isolate #1) nor Anacystis nidulans take up carbaryl readily. In the latter algae there is no uptake of label for several hours; the label ultimately taken up may represent a breakdown product (or products) of carbaryl rather than the parent compound.

2,4-DBE

While the effects of some formulations of 2,4-D on laboratory strains of algae have been studied extensively, only a few such studies have involved 2,4-DBE. Ware and Roan (1960) found that this ester of 2,4-D, at 1 mg/l, reduced $^{14}CO_2$ fixation about 15% in several phyto-

plankton species. Walsh (1972), studying the effect of 2,4-D upon the growth of four marine phytoplankton species, found that the technical acid of 2,4-D was more toxic than was the butoxy ethyl ester.

Among the significant results of the current study of interactions of 2,4-DBE and algae is the discovery that this formulation of 2,4-DBE shows a relatively low toxicity, compared to the toxicity of the other pesticides being studied. Another finding of possible significance is the minimal uptake of this formulation of 2,4-D by Chlorella sp., isolate #1, and by Anacystis nidulans. Neither organism appears to have significant binding capacity, or binding sites, for this ester form of 2,4-D.

DIAZINON

Cultures of Dunaliella euchlora and Platymonas sp. as well as natural freshwater phytoplankton communities (Butler, 1963) showed reduced $^{14}CO_2$ uptake in the presence of 1 mg/l diazinon. Estuarine phytoplankters are similarly inhibited by 1 mg/l diazinon (Ware and Roan, 1970). In contrast, in Scenedesmus quadricaudata $^{14}CO_2$ uptake is reported to be stimulated by 0.1 and 1 mg/l. Growth of the nitrogen-fixing blue-green alga Cylindrospermum sp. in N-free medium is inhibited only slightly by diazinon up to 200 mg/l. Higher levels of diazinon are inhibitory to this organism and also to Aulosira fertilissima and to Plectonema boryanum (Singh, 1973).

The effect of diazinon upon the Warrior River isolates was variable both in regards to concentrations of diazinon and to different isolates. However, at concentrations of 10 mg/l and higher most isolates were significantly inhibited. Paris et al. (1975) observed no degradation of diazinon that could be attributed to the action of mixed populations of bacteria and fungi. However, diazinon degradation has been reported in submerged soils and in rice paddies in India (Sethunathan and Yoshida, 1969; Sethunathan and Pathak, 1972). While it was determined that the degradation was biological, the microorganisms involved were not identified. It is not known whether any of the degradation should be attributed to action by soil algae in the microflora. Our studies involving small scale cultures of Warrior River isolates indicate that there is some diazinon degradation by these algae but the extent of degradation is not great.

ENDRIN

Sensitivity of algae to endrin appears to be variable. Batterton et al. (1971) found that the growth of Anacystis nidulans and Agmenellum quadriplicatum was inhibited by 0.0002 to 0.95 mg/1. Vance and Drummond (1969) reported that the LD_{100} for four unicellular algae ranged from less than 5 to more than 20 mg/1. In other tests three blue-green algae tolerated concentrations of endrin as high as 600 mg/1, although growth was less than in the absence of this insecticide (Singh, 1973). In the Warrior River isolates, several organisms were slightly inhibited at 0.01 and 0.1 mg/1; inhibition was more severe at 1.0 mg/1.

HEPTACHLOR

Little has been written about the effect of heptachlor on algae. A concentration of 60 mg/1 has been reported inhibitory to Euglena gracilis (Parrakova and Krcmery, 1967). Only a few of the Warrior River isolates show inhibition of growth, and only at the highest concentration tested, 1 mg/1.

MALATHION

Malathion in low concentration, less than 5 mg/1, appears to have little effect upon the growth of algae that have been tested so far. Moore (1970) reported a 48.9% inhibition of the growth of Euglena gracilis Z by a concentration of 7.25 mg/1. In contrast, Poorman (1973) reported growth stimulation of Euglena gracilis following a one week treatment of 10, 50 and 100 mg/1 malathion. Malathion at 100 mg/1 had little effect upon the growth of Chlorella (Christie, 1969). The effect of malathion upon nitrogen fixation by blue-green algae was studied by DaSilva et al. (1975) who found that N-fixation was initially depressed and ultimately stimulated by 100 mg/1. Concentrations of 1, 5 and 10 mg/1 malathion had no significant effect upon growth of the Warrior River isolates, but 25 and 50 mg/1 inhibited some of these organisms.

Degradation of malathion by both bacteria and fungi has been demonstrated, and degradation rates for the bacteria have been calculated (Paris et al., 1975). Studies of the loss of malathion in small-scale cultures of the Warrior River algal isolates suggested that degradation rates for algae might also be determined. The large-scale culture experiments with Chlorella sp. (isolate #1), and with Anacystis nidulans showed that malathion is degraded by these algae, and also that the degradation by Chlorella is strongly dependent upon light. The only degradation product that has been isolated and identified is the malathion β-monoacid (from the Chlorella cultures), which disappears from

the culture soon after the malathion has broken down. Degradation rates, in the light, appear to be, on a per cell basis, roughly equivalent to those reported for bacteria studied by Paris et al. (1975).

METHOXYCHLOR

There have been only limited prior studies of interactions of algae and methoxychlor. Poorman (1973) reported that methoxychlor concentrations of 50 and 100 mg/l reduced growth of Euglena gracilis, but that concentrations of 1, 5 and 10 mg/l were stimulatory. However, fixation of $^{14}CO_2$ in other phytoplanktonic algae was reported to be inhibited by 1 mg/l methoxychlor (Butler, 1963; Ware and Roan, 1970). Because the solubility of methoxychlor in water is considerably lower than these values, experiments with the Warrior River isolates were confined to those involving concentrations within the solubility range, specifically 0.001 and 0.01 mg/l methoxychlor. Methoxychlor in these concentrations stimulates growth of some isolates and inhibits growth of others.

Methoxychlor is reported to be biodegraded more rapidly than DDT (Kapoor et al., 1970; Metcalf et al. 1971). Methoxychlor can also be accumulated by bacteria from the medium to a concentration about 300 times the medium concentration (Johnson and Kennedy, 1973). Paris et al.(1975) reported that Flavobacterium harrisonii degrades methoxychlor to 2,2-bis (p-methoxyphenyl)-1,1-dichloroethylene, or methoxychlor-DDE. Mendel et al. (1967) report the same derivative when methoxychlor is degraded by Aerobacter aerogenes. Pesticide-loss studies suggested that most of the Warrior River isolates have some capacity to degrade methoxychlor. Studies with labeled methoxychlor also indicated that several isolates degrade methoxychlor, but the specific degradation products have not been identified.

Methoxychlor sorption by Chlorella pyrenoidosa has previously been reported (Paris and Lewis, 1973); under the conditions of the study, equilibrium was reached within 30 min. The Warrior River isolates tested (Chlorella sp., isolate #1 and Nitzschia sp., isolate #35), as well as Anacystis nidulans, took up significant quantities of labeled methoxychlor within 1 to 5 min, indicating physical adsorption. Furthermore, most of this labeled methoxychlor (about 90%) taken up by Chlorella sp. and by Anacystis nidulans was readily exchanged with non-labeled methoxychlor, but only about half of the labeled methoxychlor taken up by the Nitzschia isolate was exchangeable.

TOXAPHENE

Palmer and Maloney (1955) found that the effect of toxaphene, at a concentration of 2 mg/l, upon 6 unicellular algae ranged from no effect

to partial inhibition of growth. Stadnyk et al. (1971), working with Scenedesmus quadricaudata, found 0.1 mg/l toxaphene had no effect, but 1 mg/l caused a 19% decrease in cell number. The Warrior River isolates were not inhibited by 0.001 or 0.01 mg/l toxaphene; however, a significant number were inhibited by 0.1 and 1.0 mg/l. Toxaphene sorption has been demonstrated in several microorganisms, including Chlorella pyrenoidosa strain 395, and a field sample containing Scenedesmus sp. and Chlorella sp. (Paris et al., 1975).

SECTION VI

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SECTION VII

PUBLICATIONS

Aldridge, E. F., G. L. Blume, J. C. O'Kelley and T. R. Deason. Degradation of malathion by planktonic algae. In preparation.

Butler, G. L. 1974. Effects of five pesticides on twenty-one freshwater algae. Ph.D. Dissertation, University of Alabama, 1975. University, AL 35486.

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Moss, S. W., G. L. Blume, J. C. O'Kelley and T. R. Deason. Sorption and degradation of methoxychlor by planktonic algae. In preparation.

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16. ABSTRACT

In this investigation interactions of 12 pesticides with 37 strains of fresh water algae were studied in an effort to determine something of the variability in responses of fresh water algae to the variety of pesticides in use or projected to be used in the future.

Three interactions were investigated. One was the toxicity of the pesticides to these algae. Another was the sorption of several of the pesticides by some of the species of algae. The third was the possibility that some of the pesticides can be degraded by action of algae.

In general it was found that sensitivity of algae to pesticides varied greatly with the strains tested.

Sorption of methoxychlor appeared to be mainly physical, since much of the methoxychlor sorbed was exchangeable. The butoxyethyl ester of 2,4-D (2,4-DBE) was not sorbed to a significant extent by two green algae tested, and sorption of carbaryl was very slow.

Malathion can be degraded by algae in the presence of light. One breaddown proudct, malathion monoacid (beta form), appeared as the malathion was being degraded, and later disappeared. Investigations of the fate of 2,4-DBE and methoxychlor in algal cultures suggest that the fate of 2,4-DBE and methoxychlor in algal cultures suggest that these pesticides may also be degraded by algal activity.

17. KEY WORDS AND DOCUMENT ANALYSIS

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